SYNTHESIS OF 1-(3-PHTHALIMIDO-2-OXOBUTYL)-4-SUBSTITUTED-PHENYLPIPERAZINES AND THEIR ANTI-HIV REVERSE TRANSCRIPTASE ACTIVITY

CHEN Xin[1] , WANG Lin[1*] , ZHAO Zhi-zhong[1] , ZHANG Xing-quan[2] , CHEN Xiang-hong[2] , CHEN Hong-shan[2]

(*1. Institute of Materia Medica ; 2. Institute of Medicinal Biotechnology , Chinese Academy of Medical Sciences and Peking Union Medical College , Beijing 100050 , China*)

ABSTRACT: **AIM** Synthesis of 1-(3-phthalimido-2-oxobutyl)-4-substituted-phenylpiperazines (**5** ~ **15**). **METHODS** The starting material nitrogen mustard hydrochloride (**16**), reacted with the corresponding substituted anilines to afford piperazine hydrochlorides (**17** ~ **27**), which were then coupled with 1-bromo-3-phthalimidobutan-2-one (**4**) to give the target compounds. **RESULTS** Eleven target compounds (**5** ~ **15**) were synthesized, which were characterized by ^{1}HNMR, IR and elemental analysis. **CONCLUSION** Anti-HIV-1 RT using HIV reverse transcriptase P-66 protein test showed that compounds **11**, **14**, **10** and **13** possessed inhibitory effects against HIV-1 reverse transcriptase (RT), with IC_{50} 29.80, 35.20, 43.77 and 63.76 μmol·L^{-1}, respectively.

KEY WORDS: phthalimido-piperazines ; substituted phenylpiperazines ; HIV-1 RT inhibitors

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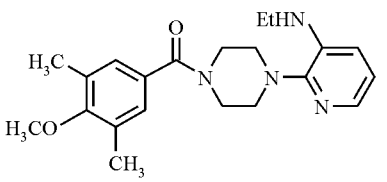
Several non-nucleoside human immunodeficiency virus reverse transcriptase (HIV-RT) inhibitors have been reported since 1991[1~5]. For example in the following compounds (**1** ~ **2**) were investigated extensively, and compound **2** (delavirdine) has been granted for sale as a new drug for the treatment of AIDS recently.



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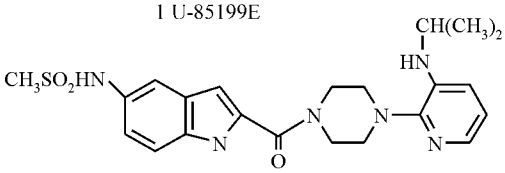
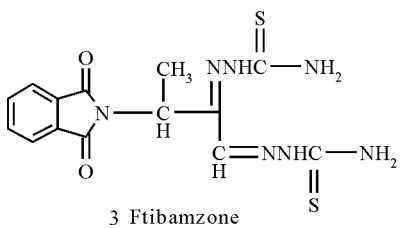


2 Delavirdine · CH_3SO_2OH

Ftibamzone (Tai-Ding-An (**3**)) is a non-nucleoside anti herpes virus drug and was synthesized by the Institute of Materia Medica, CAMS & PUMC.



3 Ftibamzone

The structure activity relationship (SAR) research of ftibamzone analogs showed that the existence of the phthalimido moiety acts as a key function to the anti-virus activity[6]. Further, Romero[5] et al. found that the saturated nitrogen-containing heterocyclic group is a pharmaco-effective group, which can inhibit HIV-1 RT. We intended to combine the two moieties and synthesized a series of new phthalimido-piperazine derivatives in order to evaluate their inhibitory activity against HIV-1 RT. We designed and synthesized the following compounds (**5** ~ **15**), and the synthetic route is shown in Scheme 1.

Diethanolamine reacted with thionyl chloride in chloroform at 70 ~ 80 ℃ to afford nitrogen mustard hydrochloride (**16**) with a yield of 91 %, which then was refluxed with different substituted anilines for 62 to 137 hours in n-butanol in the presence of base (K_2CO_3) to give piperazine hydrochlorides (**17** ~ **27**) with the yield from 15 % to 82 %, mostly 50 % ~ 60 %. The resulting hydrochlorides which were firstly neutralized with base and then coupled with 1-bromo-3-phthalimidobutan-2-one (**4**)[6] to give the target compounds (**5** ~ **15**) with

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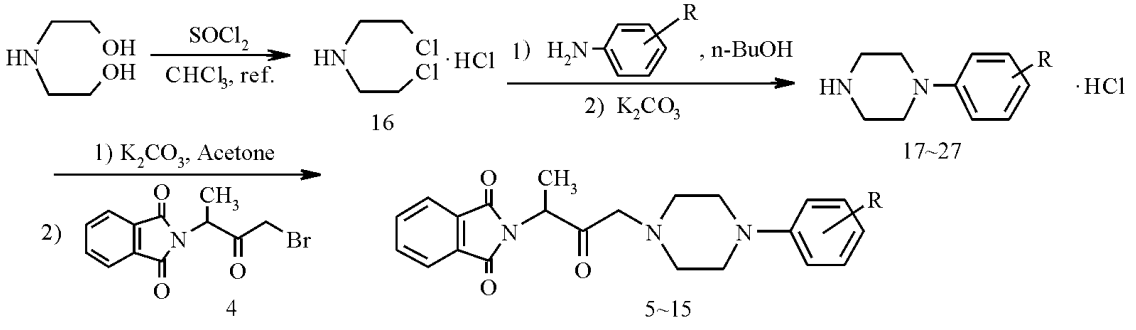
Biographies : CHEN Xin(1971 -) , male , Master of Science ; WANG Lin(1933 -) , female , Professor.

* Corresponding author Tel :(010)63165243 , Fax :(010)63017757 , E-mail :lin-wang@yeah.net

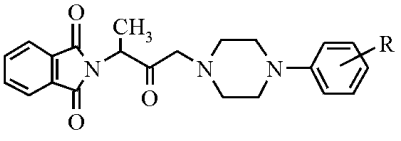
moderate to good yields (14% ~ 95%). The target compounds (**5** ~ **15**) were separated via silica gel or alumina chromatography (column or preparative TLC), depending on the substituent on the benzene ring. The structures of all compounds (**5** ~ **15**) are novel and were characterized by ^{1}HNMR, IR and elemental analysis.



R(**17** ~ **27**) : H, p-OMe (**17**, **18**) ; m-OMe, p-Cl (**19**, **20**) ; o-Cl, m-Cl (**21**, **22**) ; p-NO_2, m-NO_2 (**23**, **24**) ; p-Br, p-F (**25**, **26**) ; p-CF_3 (**27**)
R(**5** ~ **15**) : H, p-OMe (**5**, **6**) ; m-OMe, p-Cl (**7**, **8**) ; o-Cl, m-Cl (**9**, **10**) ; p-NO_2, m-NO_2 (**11**, **12**) ; p-Br, p-F (**13**, **14**) ; p-CF_3 (**15**)

Scheme 1 Route of synthesis of compounds **5** ~ **15**

Table 1 Physical properties and spectral data of compounds 5 ~ 15



No.	R	MP/℃	Yield/%[a]	IR/cm^{-1}	^{1}HNMR δ (CDCl$_3$)
5	H	128 ~ 131	27	1776, 1711, 1599, 1392	7.74(m, 4H, phthalimide-H), 6.70 ~ 7.24(m, 5H, Φ-H), 4.96(q, 1H, CH), 3.30(s, 2H, CH_2), 3.02 ~ 3.22(m, 4H, piperazinyl-H), 2.52 ~ 2.68(m, 4H, piperazinyl-H), 1.66(d, 3H, CH_3)
6	p-OMe	129 ~ 131	95	1780, 1713, 1515, 1392	7.76(m, 4H, phthalimide-H), 6.80(s, 4H, Φ-H), 4.96(q, 1H, CH), 3.72(s, 3H, OCH_3), 3.32(s, 2H, CH_2), 2.92 ~ 3.10(m, 4H, piperazinyl-H), 2.56 ~ 2.80(m, 4H, piperazinyl-H), 1.68(d, 3H, CH_3)
7[b]	o-OMe	95 ~ 100	80	1774, 1713, 1499, 1394	7.76(m, 4H, phthalimide-H), 6.90(m, 4H, Φ-H), 4.98(q, 1H, CH), 3.86(s, 3H, OCH_3), 3.30(s, 2H, CH_2), 2.90 ~ 3.20(m, 4H, piperazinyl-H), 2.50 ~ 2.80(m, 4H, piperazinyl-H), 1.68(d, 3H, CH_3)
8	p-Cl	132 ~ 133	73	1775, 1713, 1500	7.78(m, 4H, phthalimide-H), 6.40 ~ 7.62(4H, AA′BB′, Φ-H)[c], 4.92(q, 1H, CH), 3.40(s, 2H, CH_2), 3.10 ~ 3.30(m, 4H, piperazinyl-H), 2.64 ~ 2.88(m, 4H, piperazinyl-H), 1.66(d, 3H, CH_3)
9[b]	o-Cl	126 ~ 127	60	1774, 1713, 1481, 1391	7.76(m, 4H, phthalimide-H), 6.82 ~ 7.34(m, 4H, Φ-H), 4.92(q, 1H, CH), 3.70(s, 2H, CH_2), 2.90 ~ 3.44(m, 8H, piperazinyl-H), 1.66(d, 3H, CH_3)
10	m-Cl	74 ~ 75	5	1776, 1713, 1595, 1391	7.76(m, 4H, phthalimide-H), 6.60 ~ 7.12(m, 4H, Φ-H), 4.92(q, 1H, CH), 3.40(s, 2H, CH_2), 3.10 ~ 3.24(m, 4H, piperazinyl-H), 2.60 ~ 2.84(m, 4H, piperazinyl-H), 1.65(d, 3H, CH_3)
11	p-NO_2	125 ~ 127	48	1770, 1711, 1387, 1317	8.08(2H, Ar-H)[c], 7.80(m, 4H, phthalimide-H), 6.77(2H, Ar-H)[c], 4.95(q, 1H, CH), 3.36 ~ 3.54(m, 6H, CH_2 + piperazinyl-H), 2.60 ~ 2.80(m, 4H, piperazinyl-H), 1.68(d, 3H, CH_3)
12	m-NO_2	70 ~ 75	14	1776, 1713, 1526, 1389	7.76(m, 4H, phthalimide-H), 7.00 ~ 7.68(m, 4H, Φ-H), 4.86(q, 1H, CH), 3.55(s, 2H, CH_2), 3.12 ~ 3.32(m, 4H, piperazinyl-H), 2.56 ~ 2.84(m, 4H, piperazinyl-H), 1.60(d, 3H, CH_3)
13[b]	p-Br	136 ~ 138	70	1774, 1713, 1497, 1391	7.74(m, 4H, phthalimide-H), 7.24(2H, Ar-H)[c], 6.66(2H, Ar-H)[c], 4.94(q, 1H, CH), 3.32(s, 2H, CH_2), 3.00 ~ 3.16(m, 4H, piperazinyl-H), 2.52 ~ 2.68(m, 4H, piperazinyl-H), 1.60(d, 3H, CH_3)
14	p-F	104 ~ 106	40	1778, 1711, 1512, 1391	7.76(m, 4H, phthalimide-H), 6.74 ~ 6.96(4H, AA′BB′, Φ-H), 4.94(q, 1H, CH), 3.36(s, 2H, CH_2), 2.96 ~ 3.20(m, 4H, piperazinyl-H), 2.58 ~ 2.82(m, 4H, piperazinyl-H), 1.66(d, 3H, CH_3)
15[b]	p-CF_3	139 ~ 141	-[d]	1778, 1711, 1612, 1391	7.86(m, 4H, phthalimide-H), 6.84 ~ 7.56(4H, AA′BB′, Φ-H), 5.04(q, 1H, CH), 3.08 ~ 3.60(m, 6H, CH_2 + piperazinyl-H), 2.44 ~ 2.64(m, 4H, piperazinyl-H), 1.60(d, 3H, CH_3)

a. Yield calculated from piperazine hydrochlorides; b. Elemental analysis C, H, N, all within ±0.5% from calculated; c. AA′BB′ system; d. "One pot" reaction, yield calculated from compound **16** 25%

Though the intermediates of different substituted phenyl-piperazines are all known compounds , but few of them are commercially available , so we had to synthesize all the compounds **17** ~ **27** ourself . There are several methods of synthesizing monosubstituted phenyl-piperazines in literatures[7~10] , such as , nitrogen mustard react with aniline[7] , nitrogen mustard hydrochloride react with aniline hydrochloride at a high temperature[8] or the reaction of diethanolamine and aniline under catalysis of polyphosphoric acid[9] , etc . . The 1-position H-atom in the piperazine ring can also be substituted by phenyl[3] . Since substitution may occur at both sides in the 1 , 4 position of piperazine[10] , we synthesized the piperzine hydrochloride intermediates by refluxing nitrogen mustard hydrochloride with different substituted anilines in n-butanol for 62 to 137 hours , and then partly turned basic with K_2CO_3 to give the target piperazine hydrochloride intermediates (**17** ~ **27**) . The physical property data of the phenylpiperazine hydrochlorides are shown in Table 2 . We have also mentioned that , if the potassium carbonate was added in portions instead of once , the yield may increase , take the example of compound **19** , the yield was 50 % when K_2CO_3 was add once , and it raised to 82 % when potassium carbonate was added in 3 portions .

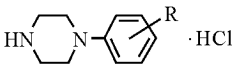
Most of the obtained target compounds are not easily crystallized . They have to be purified by silica gel chromatography . For compounds **10** and **12** ~ **15** , because they can be absorbed by silica gel , so their purification were carried out by alumina chromatography . Petroleum ether-ether (3∶1 ~ 1∶1) can be used as eluent in both case for most compounds .

The ^{1}HNMR data of compounds **5** ~ **15** are shown in Table 1 . In most instances , the peaks of the AA′ BB′ system of the aromatic H atoms of the phthalimido moiety appear in the lowest field , except for compound **11** . Due to the effect of 4-nitro group , the AA′ BB′ system of phenyl 3 ,5-position and the 2 ,6-position , the two groups of AA′ and BB′ are distant to each other and near A_2B_2 system . Further , the peaks of 3 ,5-position down shifted to the lowest field . Further , for the compounds **14** and **26** , we observed that , for the p-fluoro substituted phenyl group , the o-H spin coupled with F atom , and J = 3.6 Hz , as for the spin coupling of H (2 ,6)-F , it is not observed for the distance of the nuclears .

The IC_{50} of the active compounds are listed in Table 3 .

After screening via HIV-1 RT test of most target compounds **5** , **6** , **9** ~ **11** and **13** ~ **15** *in vitro* , the results showed that compounds **11** , **14** , **10** and **13** inhibit the activity of HIV-1 RT , with the IC_{50} of 29.80 , 35.20 , 43.77 and 63.76 μmol·L^{-1} , respectively .

Table 2 Physical properties and spectral data of compounds 17 ~ 27



No .	R	MP/ ℃	Yield/ %[a]	^{1}HNMR (DMSO-d_6)
17	H	235 ~ 238	72	9.40(D_2O exchangeable , NH) , 6.72 ~ 7.32(m ,5H , Ar-H) , 3.04 ~ 3.48(m ,8H , piperazinyl-H)
18	p-OMe	209 ~ 210	58	6.90(2H , Ar-H)[b] ,6.82(2H , Ar-H)[b] ,3.68 (s ,3H , OCH_3) ,3.21(s ,8H , piperazinyl-H) , 2.08(D_2O exchangeable , NH)
19	o-OMe	201 ~ 204	82	6.80 ~ 7.00(m ,4H , Ar-H) ,3.76(s ,3H , OCH_3) ,3.14(s ,8H , piperazinyl-H)
20	p-Cl	227 ~ 229	54	7.22(2H , Ar-H)[b] ,6.96(2H , Ar * H)[b] , 3.05 ~ 3.68(m ,8H , piperazinyl-H)
21	o-Cl	152 ~ 154	50	9.48(D_2O exchangeable , NH) ,6.96 ~ 7.48 (m ,4H , Ar-H) ,3.28(s ,8H , piperazinyl-H)
22	m-Cl	235 ~ 238	57	9.40(D_2O exchangeable , NH) , 6.74 ~ 7.32(m ,4H , Ar-H) , 3.00 ~ 3.50(s ,8H , piperazinyl-H)
23	p-NO_2	221 ~ 228 (dec .)	19	9.48(D_2O exchangeable , NH) , 8.04(2H , Ar-H)[b] ,7.05(2H , Ar-H)[b] , 3.56 ~ 3.80(m ,4H , piperazinyl-H) , 3.16 ~ 3.30(m ,4H , piperazinyl-H)
24	m-NO_2	222 ~ 228 (dec .)	51	9.20(D_2O exchangeable , NH) , 7.32 ~ 7.70(m ,4H , Ar-H) , 3.40 ~ 3.60(m ,4H , piperazinyl-H) , 3.16 ~ 3.36(m ,4H , piperazinyl-H)
25	p-Br	216 ~ 219	15	9.36(D_2O exchangeable , NH) , 7.36(2H , Ar-H)[b] ,6.90(2H , Ar-H)[b] , 3.00 ~ 3.50(s ,8H , piperazinyl-H)
26	p-F	180 ~ 184	60	9.40(D_2O exchangeable , NH) , 7.05(2H , Ar-H)[b] ,6.96(2H , Ar-H)[b] , 3.00 ~ 3.68(s ,8H , piperazinyl-H)
27	p-CF_3	> 300	11[c]	[d]

a . Yield calculated from nitrogen mustard hydrochloride ; b . AA′ BB′ system ; c . Most products remained in the reaction mixture and went directly to the next step reaction without further purification ; d . The solid was insoluble in DMSO and other solvents so there was no NMR data , but the next step to give compound **15** proved the structure

Table 3 Inhibition of compounds 5 ~ 15 on HIV-1 reverse transcriptase *in vitro*

Compound	IC_{50}/ μmol·L^{-1}	Compound	IC_{50}/ μmol·L^{-1}
5	486.26	11	29.80
6	2528.23	12	NT
7	NT	13	63.76
8	NT	14	35.20
9	126.80	15	117.91
10	43.77		

NT : Not tested

EXPERIMENT

m.p. YANACO MP-500D mp meter, thermometer uncorrected. ^{1}HNMR, Jeol FX-90Q, 90 MHz, inner standard TMS, δ in ppm, J in Hz. FT-IR, IMPACT-400. Elemental Analysis: Carlo-Erba 1106.

1 Synthesis of nitrogen mustard hydrochloride (16)

Conducted via ordinary method, yield 91 %. mp 207 ~ 209 ℃.

2 Synthesis of different substituted-phenylpiperazines (17 ~ 27)

Reaction example, phenylpiperazine hydrochloride (**17**). To a solution of 0.526 g (3 mmol) of nitrogen mustard hydrochloride (**16**) in 15 mL of n-butanol, 0.280 g (3 mmol) of redistilled aniline and 0.069 g (0.5 mmol) of potassium carbonate (K_2CO_3) were added therein. The resultant reaction mixture was refluxed for 8 hours in a dry atmosphere and then cooled to room temperature. After that, 69 mg (0.5 mmol) of K_2CO_3 was added and the mixture was refluxed for 8 hours and cooled to room temperature. Then another 69 mg (0.5 mmol) of K_2CO_3 was added and the mixture was refluxed for another 7 hours. At the end point that no much bubbles appeared, the following procedures based on the reference[10], 410 mg (72 %) of the title compound was obtained as white crystal, mp 235 ~ 238 ℃ (ref.[8] 248 ℃), elemental analysis $C_{10}H_{15}ClN_2 \cdot \frac{1}{4}H_2O$, Calc (%): C 59.11, H 7.69, N 13.79; Found (%): C 59.64, H 7.55, N 13.63.

The syntheses of compounds (**18 ~ 27**) were similar to compound (**17**).

3 Synthesis of phthalimidopiperazines with different substituted phenyl substituents (5 ~ 15)

Reaction example, synthesis of 1-(3-phthalimido-2-oxobutyl)-4-(2-chlorophenyl) piperazine (**9**) 175 mg (0.75 mmol) of o-chlorophenyl-piperazine hydrochloride (**21**) was stirred with 0.625 g (4.5 mmol) of K_2CO_3 in dry acetone until the base of **21** liberated, and then 300 mg (1 mmol) of 1-bromo-3-phthalimido-2-butanone (**4**) was added therein. The reaction mixture was stirred under ambient temperature, and controlled with TLC (ether-petroleum ether = 3∶2) until the reaction completed. The inorganic salt was filtered off, the filtrate was concentrated to dry under reduced pressure and then uploaded to 0.7 g of silica gel (200 ~ 300 mesh grade), separated with basic alumina (5.5 g) chromatography (ether-petroleum ether = 1∶3), 190 mg of title compound was obtained as white sheet crystal (mp 126 ~ 127 ℃, yield 60 %), elemental analysis $C_{22}H_{22}ClN_3O_3$ calc (%): C 64.15, H 5.38, N 10.20; found (%): C 64.36, H 5.30, N 10.03.

The synthesis processes of the rest compounds (**5 ~ 8, 10 ~ 15**) were similar to the above description.

4 Inhibition of HIV-1 Reverse transcriptase

The HIV reverse transcriptase P-66 protein was used to test the inhibitory activities of compounds with the method reported by Tang, et al in 1990[12].

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1-(3-酞酰亚胺基-2-氧丁基)-4-取代苯基哌嗪的合成及抗 HIV-1 逆转录酶活性

陈 昕[1] , 王 琳[1] , 赵知中[1] , 陈湘红[2] , 张兴权[2] , 陈鸿珊[2]

(中国医学科学院 ,中国协和医科大学 1 . 药物研究所 ; 2 . 医药生物技术研究所 , 北京 100050)

摘要 : 目的 合成新型的非核苷类(双杂环苯基) 化合物 ,并观察其抗 HIV1-逆转录酶(HIV1-RT) 活性 。方法 以氮芥盐酸盐为起始原料 ,与不同取代苯胺反应 ,得到相应的不同取代的哌嗪盐酸盐 ,并与 1-溴-3-酞酰亚胺基-2-丁酮(**4**) 缩合 ,得到目标化合物 。结果 合成 11 个目标化合物(**5** ~ **15**) 。经[1] HNMR ,红外和元素分析确定结构 。结论 经 HIV 逆转录酶 P-66 蛋白测定 ,化合物 **11** ,**14** ,**10** 和 **13** 有一定抑制 HIV1-RT 活性 ,其 IC_{50} 分别为 29.80 ,35.20 ,43.77 和 63.76 μmol·L^{-1} 。

关键词 : 酞酰亚胺基哌嗪 ; 取代苯基哌嗪 ; HIV1-逆转录酶抑制剂